Development & Performance Assessment of a New ATMP for Cartilage Tissue Engineering

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Introduction

Joint disorders are responsible of reduced quality of life and high socio-economic burden in our progressively ageing and physically active population. Even though huge efforts on the development of cartilage repair strategies, presently no approach has demonstrate full efficiency. The present study aimed to create an innovative advanced-therapy medicinal product (ATMP) where human adipose-derived stem/stromal cells (hASCs) [1] encapsulated within a novel hydrogel based on methacrylated gellan gum (mimsys G) [2] will develop cartilage-like tissue. These complete xeno-free 3D approach was developed and *in vitro* experiment were conducted under static and dynamic conditions, the latest using a rotational dual chamber bioreactor [3]. Preliminary *in vivo* assay using rabbit model of cartilage defect was based on autologous and allogenic approaches. Both *in vitro* and *in vivo* experiments were established to evaluate the performance of this system on focal cartilage tissue engineering.

Materials and Methods

Human ASC (hASC xeno-free, irisbiosciences), isolated in GMP conditions, were expanded in xeno-free media until sub-confluency. mimsys G hydrogel (mimsys®G, irisbiosciences) was prepared to yield a 2% w/V solution. Cells were encapsulated at $10x10^6$ cells/mL, ionically crosslinked and cultured with or without chondrogenic induction for 21 days. For dynamic growth, a rotational dual chamber bioreactor was used to control the perfusion and flow gradient within the cellular scaffolds. Cell viability was determined by live/dead assay, and cell metabolic activity by MTS reduction. A *in vivo* study evaluated regeneration of focal cartilage lesions on a rabbit knee model for 8 weeks, using autologous and allogenic approaches. Rabbit ASCs (rASCs), isolated from fat pad, and hASCs were encapsulated into mimsys G prior administration into the cartilage defect. Chondrogenesis was assessed on both *in vitro* and *in vivo* samples by histological staining of cartilage matrix using safranin O and immunolocalization of collagen type II. Expression of chondrogenic-related genes (aggrecan, collagen type II and sox-9) were also determined.

Results

In vitro analysis of the new ATMP demonstrated high viability of the cells and progressive metabolic activity along static and dynamic culture. Additionally, intense cartilage matrix deposition was detected through up-regulation of chondrogenic maker Collagen II gene and protein (Immunohistochemistry) by end of culture (Fig.1). For the *in vivo* study, both autologous and allogenic approaches demonstrated the establishment of lateral and subchondral integration (Fig.2).

Fig. 1

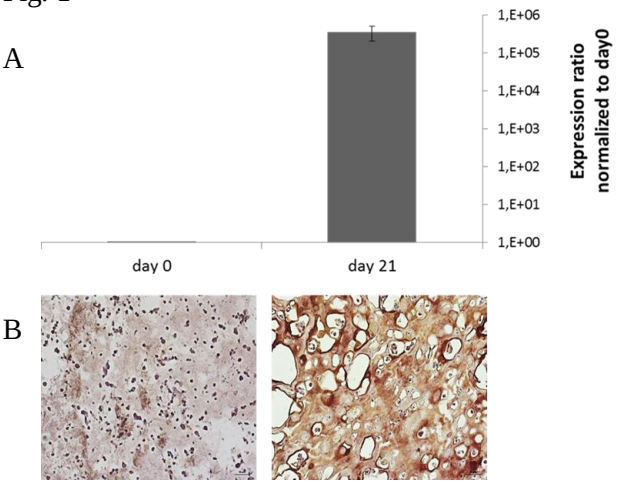

Fig. 2

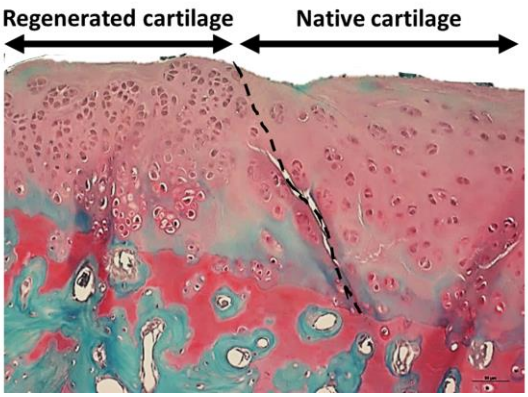

Fig.1. *In vitro* Chondrogenesis – Collagen II gene (A) and protein (B) expression at day 0 and day 21, after chondrogenic induction in static culture conditions. Fig 2. *In vivo* study – Safranin-O staining after regeneration of cartilage lesion.

Discussion and Conclusions

The proposed innovative ATMP demonstrated intense cartilage development *in vitro* through evaluation of specific markers. Preliminary *in vivo* data demonstrates promising repair performance in focal cartilage lesions, suggesting a valuable approach for further therapeutic exploitation.

References

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